

What is claimed is:

1. A mixed sequence oligonucleotide comprising at least 12 nucleotides in length and having a 3' end and a 5' end and divided into a first portion and a further portion,
5 said first portion being capable of supporting cleavage of a complementary target RNA by human RNase H1 polypeptide,
 said further portion being incapable of supporting said cleavage by said RNase H1;
10 wherein said first portion comprises at least 6 nucleotides and is positioned in said oligonucleotide such that at least one of said 6 nucleotides is 8 to 12 nucleotides from the 3' end of said oligonucleotide.
2. The oligonucleotide of claim 1 comprising at
15 least one CA nucleotide sequence within said first portion.
3. The oligonucleotide of claim 1 comprising from about 12 to about 50 nucleotides.
4. The oligonucleotide of claim 1 comprising from about 12 to about 25 nucleotides.
- 20 5. The oligonucleotide of claim 1 wherein each of said nucleotides of said first portion have B-form conformational geometry and are joined together in a continuous sequence.
- 25 6. The oligonucleotide of claim 1 wherein each of said nucleotides of said first portion is, independently, a 2'-deoxyribonucleotide, a 2'-SCH₃ ribonucleotide, a 2'-NH₂ ribonucleotide, a 2'-NH(C₁-C₂ alkyl) ribonucleotide, a 2'-N(C₁-C₂ alkyl)₂ ribonucleotide, a 2'-CF₃ ribonucleotide, a

2'=CH₂ ribonucleotide, a 2'=CHF ribonucleotide, a 2'=CF₃ ribonucleotide, a 2'-CH₃ ribonucleotide, a 2'-C₂H₅ ribonucleotide, a 2'-CH=CH₂ ribonucleotide or a 2'-C≡CH ribonucleotide.

5 7. The oligonucleotide of claim 1 wherein each of said nucleotides of said first portion is a 2'-deoxyribonucleotide.

8. The oligonucleotide of claim 1 wherein each of said nucleotide of said first portion is, independently, a
10 2'-CN arabinonucleotide, a 2'-F arabinonucleotide, a 2'-Cl arabinonucleotide, a 2'-Br arabinonucleotide, a 2'-N₃ arabinonucleotide, a 2'-OH arabinonucleotide, a 2'-O-CH₃ arabinonucleotide or a 2'-dehydro-2'-CH₃ arabinonucleotide.

9. The oligonucleotide of claim 1 wherein each of
15 said nucleotides of said first portion is, independently, a 2'-F arabinonucleotide, a 2'-OH arabinonucleotide or a 2'-O-CH₃ arabinonucleotide.

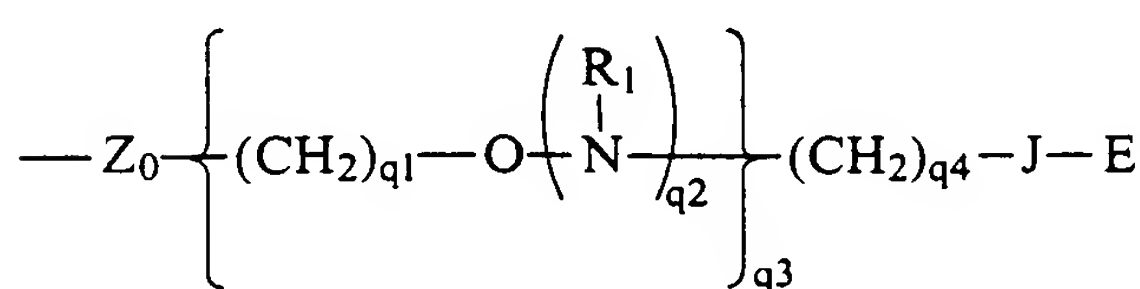
10. The oligonucleotide of claim 1 wherein each of said nucleotides of said first portion is, independently, a
20 2'-F arabinonucleotide or a 2'-OH arabinonucleotide.

11. The oligonucleotide of claim 1 wherein said nucleotides of said first portion are joined together in said continuous sequence by phosphate, phosphorothioate, phosphorodithioate or boranophosphate linkages.

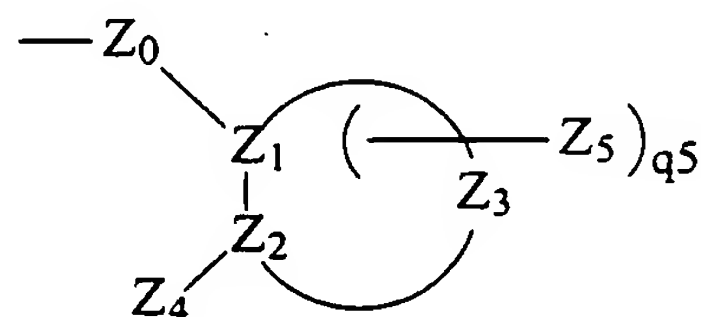
25 12. The oligonucleotide of claim 1 wherein said further portion includes a plurality of nucleotides, at least some of said nucleotides comprise a 2' substituent group wherein each substituent group is, independently,

hydroxyl, C₁-C₂₀ alkyl, C₂-C₂₀ alkenyl, C₂-C₂₀ alkynyl, halogen, amino, thiol, keto, carboxyl, nitro, nitroso, nitrile, trifluoromethyl, trifluoromethoxy, O-alkyl, O-alkenyl, O-alkynyl, S-alkyl, S-alkenyl, S-alkynyl, NH-alkyl, NH-alkenyl, NH-alkynyl, N-dialkyl, O-aryl, S-aryl, NH-aryl, O-aralkyl, S-aralkyl, NH-aralkyl, N-phthalimido, imidazole, azido, hydrazino, hydroxylamino, isocyanato, sulfoxide, sulfone, sulfide, disulfide, silyl, aryl, heterocycle, carbocycle, intercalator, reporter molecule, conjugate, polyamine, polyamide, polyalkylene glycol, or polyether;

or each substituent group has one of formula I or II:



I



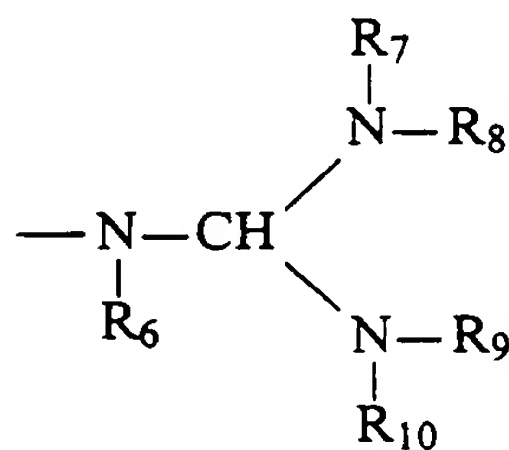
II

wherein:

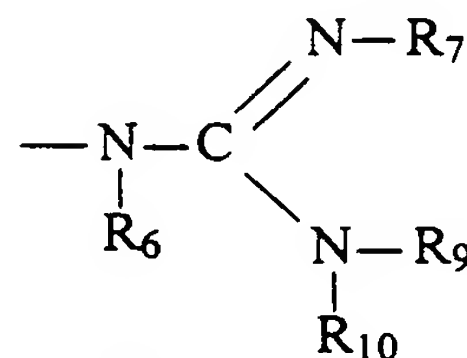
Z₀ is O, S or NH;

J is a single bond, O or C(=O);

E is C₁-C₁₀ alkyl, N(R₁)(R₂), N(R₁)(R₅), N=C(R₁)(R₂), N=C(R₁)(R₅) or has one of formula III or IV;



III



IV

each R₆, R₇, R₈, R₉ and R₁₀ is, independently, hydrogen, C(O)R₁₁, substituted or unsubstituted C₁-C₁₀ alkyl, substituted or unsubstituted C₂-C₁₀ alkenyl, substituted or unsubstituted C₂-C₁₀ alkynyl, alkylsulfonyl, arylsulfonyl, a

chemical functional group or a conjugate group, wherein the substituent groups are selected from hydroxyl, amino, alkoxy, carboxy, benzyl, phenyl, nitro, thiol, thioalkoxy, halogen, alkyl, aryl, alkenyl and alkynyl;

5 or optionally, R_7 and R_8 , together form a phthalimido moiety with the nitrogen atom to which they are attached;

or optionally, R_9 and R_{10} , together form a phthalimido moiety with the nitrogen atom to which they are attached;

each R_{11} is, independently, substituted or

10 unsubstituted C_1 - C_{10} alkyl, trifluoromethyl, cyanoethyloxy, methoxy, ethoxy, t-butoxy, allyloxy, 9-fluorenylmethoxy, 2-(trimethylsilyl)-ethoxy, 2,2,2-trichloroethoxy, benzyloxy, butyryl, iso-butyryl, phenyl or aryl;

R_5 is T-L,

15 T is a bond or a linking moiety;

L is a chemical functional group, a conjugate group or a solid support material;

each R_1 and R_2 is, independently, H, a nitrogen protecting group, substituted or unsubstituted C_1 - C_{10} alkyl, 20 substituted or unsubstituted C_2 - C_{10} alkenyl, substituted or unsubstituted C_2 - C_{10} alkynyl, wherein said substitution is OR_3 , SR_3 , NH_3^+ , $N(R_3)(R_4)$, guanidino or acyl where said acyl is an acid amide or an ester;

or R_1 and R_2 , together, are a nitrogen protecting group 25 or are joined in a ring structure that optionally includes an additional heteroatom selected from N and O;

or R_1 , T and L, together, are a chemical functional group;

each R_3 and R_4 is, independently, H, C_1 - C_{10} alkyl, a 30 nitrogen protecting group, or R_3 and R_4 , together, are a nitrogen protecting group;

or R_3 and R_4 are joined in a ring structure that optionally includes an additional heteroatom selected from N and O;

Z_4 is OX, SX, or $N(X)_2$;

each X is, independently, H, C_1-C_8 alkyl, C_1-C_8 haloalkyl, $C(=NH)N(H)R_5$, $C(=O)N(H)R_5$ or $OC(=O)N(H)R_5$;

R_5 is H or C_1-C_8 alkyl;

5 Z_1 , Z_2 and Z_3 comprise a ring system having from about 4 to about 7 carbon atoms or having from about 3 to about 6 carbon atoms and 1 or 2 hetero atoms wherein said hetero atoms are selected from oxygen, nitrogen and sulfur and wherein said ring system is aliphatic, unsaturated
10 aliphatic, aromatic, or saturated or unsaturated heterocyclic;

Z_5 is alkyl or haloalkyl having 1 to about 10 carbon atoms, alkenyl having 2 to about 10 carbon atoms, alkynyl having 2 to about 10 carbon atoms, aryl having 6 to about
15 14 carbon atoms, $N(R_1)(R_2)OR_1$, halo, SR_1 or CN;

each q_1 is, independently, an integer from 1 to 10;

each q_2 is, independently, 0 or 1;

q_3 is 0 or an integer from 1 to 10;

q_4 is an integer from 1 to 10; and

20 q_5 is from 0, 1 or 2;

provided that when q_3 is 0, q_4 is greater than 1.

13. The oligonucleotide of claim 1 wherein each of said nucleotides of said further portion is, independently, a 2'-F ribonucleotide, a 2'-O-(C_1-C_6 alkyl) ribonucleotide,
25 or a 2'-O-(C_1-C_6 substituted alkyl) ribonucleotide wherein the substitution is C_1-C_6 ether, C_1-C_6 thioether, amino, amino(C_1-C_6 alkyl) or amino(C_1-C_6 alkyl)₂.

14. The oligonucleotide of claim 1 wherein said
30 nucleotides of said further portion are joined together in a continuous sequence by 3'-5' phosphodiester, 2'-5' phosphodiester, phosphorothioate, Sp phosphorothioate, Rp phosphorothioate, phosphorodithioate, 3'-deoxy-3'-amino

phosphoroamidate, 3'-methylenephosphonate, methylene(methylimino), dimethylhydrazino, amide 3, amide 4 or boranophosphate linkages.

15. The oligonucleotide of claim 1 wherein at least
5 two of said nucleotides of said further portion are joined together in a continuous sequence that is positioned 3' to said first portion.

16. The oligonucleotide of claim 1 wherein at least
10 two of said nucleotides of said further portion are joined together in a continuous sequence that is positioned 5' to said first portion.

17. The oligonucleotide of claim 1 wherein at least
two of said nucleotides of said further portion are joined together in a continuous sequence that is positioned 3' to
15 said first portion and at least two of said further portion are joined together in a continuous sequence that is positioned 5' to said first portion.

18. The oligonucleotide of claim 1 wherein at least
four of said nucleotides of said further portion are joined
20 together in a continuous sequence that is positioned 3' to said first portion.

19. The oligonucleotide of claim 1 wherein at least
four of said nucleotides of said further portion are joined together in a continuous sequence that is positioned 5' to
25 said first portion.

20. The oligonucleotide of claim 1 wherein at least
four of said nucleotides of said further portion are joined together in a continuous sequence that is positioned 3' to

said first portion and at least four of said nucleotides of said further portion are joined together in a continuous sequence that is positioned 5' to said first portion.

21. A mixed sequence oligonucleotide comprising at least 8 nucleotides and having a CA nucleotide sequence wherein at least one of the two nucleotides of said CA sequence is positioned 8 to 12 nucleotides from the 3' end of said oligonucleotide.

22. The oligonucleotide of claim 21 wherein said oligonucleotide is capable of supporting cleavage of a complementary target RNA by human RNase H1 polypeptide.

23. A mixed sequence chimeric oligonucleotide comprising at least 8 nucleotides and having a CA nucleotide sequence wherein at least one of the two nucleotides of said CA sequence is positioned 8 to 12 nucleotides from the 3' end of said oligonucleotide.

24. The chimeric oligonucleotide of claim 23 wherein said oligonucleotide is capable of supporting cleavage of a complementary target RNA by human RNase H1 polypeptide.

25. A mixed sequence oligonucleotide comprising 8 to 25 nucleotides and having a CA nucleotide sequence wherein at least one of the nucleotides of said CA sequence is positioned 8 to 12 nucleotides from the 3' end of said oligonucleotide.

26. A mixed sequence chimeric oligonucleotide comprising 8 to 25 nucleotides and having a CA nucleotide sequence wherein at least one of the nucleotides of said CA sequence is positioned 8 to 12 nucleotides from the 3' end

of said oligonucleotide.

27. A chimeric oligonucleotide comprising 8 to 25 nucleotides and having a portion capable of supporting cleavage of a complementary target RNA by human RNase H1 polypeptide wherein said portion supporting said cleavage is at least 6 nucleotides in length and is positioned in said oligonucleotide such that at least one of said 6 nucleotides is positioned 8 to 12 nucleotides from the 3' end of said oligonucleotide.

28. The oligonucleotide of claim 27 wherein said oligonucleotide comprises at least one CA nucleotide sequence within said portion supporting said cleavage.